APPENDIX B – COMPLETE SET OF PENDING CLAIMS

12. (Twice amended) Non-human mammalian embryonic stem cells containing a nucleic acid construct comprising a mammalian germline-specific promoter operatively associated with a recombinase coding sequence, wherein the nucleic acid construct is in the genome of the stem cells and wherein the recombinase is not expressed in the stem cells in cell culture.

13. (Amended) Embryonic stem cells according to claim 12 wherein the genome thereof further comprises a transcriptionally active selectable marker flanked by two recombinase recombination target sites.

14. (Amended) Embryonic stem cells according to claim 13 wherein the recombinase encoded by the recombinase coding sequence is selective for the recombination target sites flanking said selectable marker.

15. (Reiterated) Embryonic stem cells according to claim 13 further comprising one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

18. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes Cre recombinase.

19. (Amended) Embryonic stem cells according to claim 18 wherein said construct is ProCre, comprising the protamine 1 gene promoter operatively associated with Cre recombinase.

20. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes FLP recombinase.

21. (Amended) Embryonic stem cells according to claim 20 wherein said construct is ProFLP, comprising the protamine 1 gene promoter operatively associated with FLP recombinase.

22. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes the R gene product of *Zygosaccharomyces*.

23. (Amended) Embryonic stem cells according to claim 22 wherein said construct is ProR, comprising the protamine 1 gene promoter operatively associated with the R gene product of *Zygosaccharomyces*.

24. (Twice amended) Embryonic stem cells according to claim 12 further comprising an inducible promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

26. (Thrice Amended) Non-human mammalian embryonic stem cells comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

28. (Thrice Amended) A method for excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 26, said method comprising:

passaging the genome derived from said embryonic stem cells through gametogenesis, wherein said passaging causes excision of the transcriptionally active selectable marker.

29. (Reiterated) A method according to claim 28 wherein said genome is passaged through spermatogenesis.

30. (Reiterated) A method according to claim 28 wherein said genome is passaged through oogenesis.

31. (Reiterated) A method according to claim 28 wherein said embryonic stem cells further comprise one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

32. (Thrice Amended) A method for the production of recombinant alleles in a transgenic non-human animal, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombinase recombination target sites into mammalian embryonic stem cells of claim 12; and

passaging the genome derived from said embryonic stem cells through gametogenesis to obtain a transformed gamete; and

obtaining progeny from the transformed gamete, thereby producing a transgenic non-human animal having a recombinant allele therein.

34. (Reiterated) A method according to claim 32 wherein said nucleic acid fragment is introduced by homologous recombination, random insertion, retroviral insertion, or site specific-mediated recombination.

35. (Thrice Amended) A method for the production of recombinant alleles in a rodent, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells of claim 26, wherein said cells are rodent cells,

passaging the genome derived from said embryonic stem cells through gametogenesis without causing recombination of the recombination target sites,

producing offspring resulting from crossing the genome of a gamete produced by the gametogenesis with the genome of a wild type rodent,

whereby the nucleic acid fragment is inserted into the genome of the offspring and produces the recombinant allele therein.

36. (Reiterated) A method according to claim 35 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

37. (Reiterated) A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in response to inducing conditions.

38. (Reiterated) A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in a tissue selective manner.

39. (Reiterated) A method according to claim 35 wherein the recombination target sites flanking said nucleic acid fragment are recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

40. (Thrice Amended) A method for the production of recombinant alleles, said method comprising:

introducing at least one nucleic acid construct into the genome of mammalian embryonic stem cells,

wherein said at least one nucleic acid construct comprises a germline-specific promoter operatively associated with a recombinase coding sequence, a nucleic acid fragment flanked by a first pair of recombination target sites and a selectable marker flanked by a second pair of recombination target sites,

passaging the genome derived from embryonic stem cells selected for expression of the marker through gametogenesis to obtain a transformed gamete; and

crossing the genome of the transformed gamete with the genome of a wild type animal, thereby obtaining first generation progeny wherein the marker is excised in the germline.

41. (Amended) A method according to claim 40 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germline-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

42. (Reiterated) A method according to claim 40 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

43. (Thrice Amended) A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombinase recombination target site, and wherein at least one of said segments contains at least two recombinase recombination target sites,

said method comprising:

introducing said individual inactive gene segments into a mammalian embryonic stem cell of claim 12, wherein recombinase is expressed, thereby producing a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said embryonic stem cells through gametogenesis.

44. (Thrice Amended) A method for the generation of recombinant non-human animal, said method comprising:

combining a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence with host pluripotential ES cells derived from early preimplantation embryos,

introducing these embryos into a host female, and

allowing the derived embryos to come to term such that a recombinant non-human animal is thereby produced by operation of the recombinase upon passage of the genome derived from the embryonic stem cell through gametogenesis.

46. (New) The method according to claim 32 wherein the non-human animal is a rodent.

47. (New) The method according to claim 46 wherein the rodent is a mouse.

48. (New) The method according to claim 35 wherein the rodent is a mouse.

49. (Amended) The cells according to claim 12 wherein the non-human mammalian embryonic stem cell is a rodent cell.

50. (Amended) The cells according to claim 49 wherein the rodent is a mouse.

51. (Amended) The cells according to claim 12 wherein the non-human mammalian embryonic stem cell is a livestock stem cell.